Cellulose Fibre Identification through Color Vectors of Stained Fibre

Michal Jablonský,[a,*] Lenka Dubinyová,[a] Štefan Varga,[b] Katarína Vizárová,[a] Jozef Šima,[c] and Svetozár Katuščák [a]

Simple, rapid staining analytical methods with visual color assessment are the most used in practice, but they involve problems including subjective error, low accuracy, qualitative results, and the necessity of using many kinds of stains and a great deal of morphology information for correct identification. A method of objective fiber identification using color vectors of a microscan from stained fibre digital photography is described in this report. A model set of cellulose fibres was prepared: groundwood; sulphate pulp; Whatman paper; and rag fibres. The objective micro-colorimetric method, using RGB (red, green, blue) vectors with discriminatory analysis, reduced the number of stains to 1; requires no morphological information; and the discriminatory power (d_p,) of this approach is up to 95 to 100% of correctly identified unknown samples with one color vector R or B. A d_p value of 100% was achieved when using 2-P or 3-P combinations of R, G, and B.

Keywords: Cellulose; Fibre identification; Objective method; Staining; Colorimetry; RGB; Discriminatory analysis; Optical microscopy

Contact information: a: Slovak University of Technology, Faculty of Chemical and Food Technology, Institute of Natural and Synthetic Polymers, Department of Wood, Pulp and Paper, Radlinského 9, Bratislava, 812 37, Slovak Republic; b: Institute of Information Engineering, Automation, and Mathematics, Radlinského 9, Bratislava, 812 37, Slovak Republic; c: Department of Inorganic Chemistry, Radlinského 9, Bratislava, 812 37, Slovak Republic;
** Corresponding author: michal.jablonsky@stuba.sk*

INTRODUCTION

There are two groups of fibre identification methods, separated in terms of availability, cost, and practical use: (1) methods using complex scientific devices (Chen *et al.* 1996; Durán and Angelo 1998; IWTO-58 2000; Bergfjord and Holst 2010; Rezič *et al.* 2010; Paolella *et al.* 2013) and (2) methods using subjective sensory evaluation of their form, smell, staining, and color (ČSN 1976; Goodway 1987; STN 1993; ASTM 2007; TAPPI 2008).

Staining analytical methods are simple, rapid, low-cost, available, and are the most frequently used in fibre identification. However, they face problems including subjective error and low accuracy. In spite of the subjectivity and lack of quantitative data, staining methods do not require complex equipment and are therefore widely used in industries utilizing polymeric materials such as wood, pulp, and paper (Parham and Gray 1990; Franke 1993; Durán and Angelo 1998; Meng *et al.* 2013; Chen *et al.* 2015; Liu *et al.* 2015); fibre and textiles (Perry *et al.* 1985); the automotive industry (Abendshien *et al.* 2011); cultural heritage protection (Goodway 1987); archeology (Chen *et al.* 1996; Anheuser and Roumeliotou 2003); forensic science and criminal investigation (Neel and McIntyre 2010); medicine (Kumar and Gill 2010; Kostrominova

et al. 2013); developmental biology and the study of renewing tissues (Gavrieli *et al.* 1992); the food industry (Jit 1995); agriculture (AATCC 1990); and for development of modern opto-electronic methods and instruments (Suslick *et al.* 2002; Askim *et al.* 2013).

The problem with these methods is the objectivisation of simple staining methods using discriminatory analysis of generally available RGB (red, green, blue) data from the picture elements of stained fibre microphotographs. Possibilities of reducing the number of stains, dimensionality, and data volume and increasing discriminatory power are being searched for.

To make fibre identification more objective, Herzberg stain (zinc chloride-iodine stain) was selected to stain the fibres. The reasons for this choice are as follows: (1) the stain is recommended by ISO, TAPPI, ASTM, and corporate standards (ČSN 1976; STN 1993; ASTM 2007; TAPPI 2008) for the identification of the cellulose model fibres used in our model; (2) it is especially suitable for the chosen cellulose fiber model system; (3) it is used in micro- and macroscopic diagnostics (Dogu and Grabner 2010); and (4) our preliminary tests have shown that it is possible to reduce the dimensionality and number of stains while simultaneously achieving a high percentage of correctly identified unknown samples. The number of stains recommended is sometimes 2, 3, or more (ČSN 1976; STN 1993; ASTM 2007; TAPPI 2008) which would increase data volume, dimensionality, testing time, and the cost of objective analyses.

The triiodide group of Herzberg stains—Graff "C" stain, Loffton-Meritte stain, and phloroglucinol—were used in this study in preliminary testing. These stains cannot be applied to wet plant fibers because any dilution of the stain decreases its stability. They are also light-sensitive and easily oxidized so that they must be stored in a refrigerator in small quantities in stoppered, dark glass bottles. A solution suitable for treating wet fibres is a stain made from a solution of potassium iodide and iodine mixed with phosphoric acid just prior to use (Walker and Bullwinkel 1989).

The coloration of fibres depends on the chemical nature of fibre, on the fine structure of the fibre, and chemical modification. The depth of color is dependent upon the type and degree of cooking (Woodward 2002). Simons (1950) described a two-color differential stain for investigation of fibrillation and mechanical damage of beaten fibre. After beating, the fibrils and fibre debris and bruised spots in the fibres stained orange, and unbeaten fibres appeared blue (Yu *et al.* 1995). Colorability can affect the particle size of dyes, since smaller particles can penetrate very small capillaries. This can cause changes in colorability. The results of these experiments indicated that the staining response of the fibres is dependent on the proportion of pores of certain sizes (Yu *et al.* 1995; Fernando and Daniel 2010). When the fibre wall is made more accessible by beating or other means, the yellow dye can penetrate the largest pores and displace the blue dye because of its higher affinity for cellulose (Simons 1950; Yu *et al*. 2002). Katuscak and Katuscakova (1987) revealed that coloring the wood with pH indicators can be used for the identification of decay in spruce wood.

Instead of using 2 or more stains (ČSN 1976; STN 1993; ASTM 2007; TAPPI 2008), only a single zinc chloride-iodine stain was used in this work, as selected by preliminary experimental testing. The key questions addressed in this report are: (1) what would be the advantages of objectivisation using RGB vectors from generally available digital photography or fibre scans for fibre material identification; (2) can the number of stains can be reduced; and (3) what is the discriminatory power of the individual color vectors R, G, B, and their combinations, both in statistical tests and experimentally measured, by the percentage of the correctly identified unknown samples?

EXPERIMENTAL

Materials

Tissue preparation

The model set of fibres studied were F1, groundwood; F2, sulphate pulp (bleached); F3, Whatman paper; and F4, rag fibres consisting mainly of cotton. Each kind of fibrous material was prepared by defibrating according to standards (ČSN 1976; STN 1993; ASTM 2007; TAPPI 2008).

Reagents

The zinc chloride-iodine stain (Herzberg stain) and the stain prepared from aluminium, calcium, and zinc chlorides, together with iodine and potassium iodide (Graff "C" stain), were prepared according to standards (ČSN 1976; STN 1993; ASTM 2007; TAPPI 2008).

Staining

The Herzberg stain was chosen for the objective colorimetric identification of stained fibres because of its efficacy, simplicity, and the fact that it is especially useful to differentiate between rag, groundwood, and chemical wood pulps. The defibrated groundwood, sulphate pulp, Whatman paper, and rag fibres were taken by a micropipette and placed on glass slides, and the fibres were evenly distributed by a preparation needle. The microscopic slide was heated and dried completely. The dye was applied, and the fibers were covered with a coverslip without creating air bubbles. They were allowed to stand for 1 to 2 min and the excess dye was soaked up with absorbent paper.

Methods

Visual assessment

The microscopic preparations were stained with Herzberg and Graff "C" dyes. After staining, the fibers were divided based on a subjective visual assessment of the color and the standard color chart for iodine stain. Visual observation and assessment was made at 40- to 100-times magnification according to various standards (ČSN 1976; STN 1993; ASTM 2007; TAPPI 2008). The microphotographs for microcolorimetric cellulose fibre identification were taken at 40x magnification (Table 1).

Taking a microphotographic image and scans

To track the microscopic slides, an MIC 456 optical microscope with an Olympus Camedia Digital Camera C-5050 ZOOM with a 1/1.8" (7.18 x 5.32 mm) 5 megapixel CCD sensor was used. Microphotographic images were generated at 40x magnification. In the microphotograph of the sample, one fibre was selected and the area of one scan (A_s) was marked perpendicular to the fibre length as measured using a cursor; the width of each scan was 7 picture elements (pixels, px) and the area, A_s, was 7-times w_f (px), where w_f was the fibre width and was between 123 and 487 pixels or 814 and 3222 μm; the number of pixels in one scan, n_{px}, varied in the range of 861 to 3409. The area of the scans of the cellulose fibre microphotographs used in this work, at 40-fold magnification, to the area of real sample, $A_{s,r}$ (mm^2), ranged from 0.04 to 0.15. The scanned data (R, G, and B) from each picture element (pixel) were transferred to a reference database such that the R, G, and B measured data, and any derived data for each pixel, correspond to one line in the database.

Reference

To form a reference data set for the cellulose fibre types, three images were selected from each type of fibre denoted F1 to F4. In each microimage, one fibre was chosen and the scanning was performed as described previously.

For the reference set: 3 images, 3 scans, npx <861, 3409> were used per 1 fibre type. Subsequently from these pixels, 100 px were selected at random from each type of fibre, and used as reference for identification of each unknown sample by discriminant analysis.

To evaluate the homogeneity of the selected set of data and the mutual distinguishability of the types and extent of internal correlations of groups F1 to F4, statistical methods, ANOVA, MANOVA, correlation analysis, and evaluation of the extent of mutual distinctiveness of individual type pairs F1 to F4 were conducted. Identification of an unknown fibre sample was carried out by discriminatory analysis of the RGB values of the sample and those of the reference sets F1 to F4 (Varga 2012).

Identification of unknown fibre kind sample.

An unknown sample delivered for identification can be either a physical sample or an image of stained fibres prepared from an analysed fibre material. The physical unknown sample should be defibrated, stained, and microscopically observed as described above. Three microscopic images were prepared from each unknown sample; 1 scan ($A_s = 7 \times w_f$) was taken from each image as described above.

Estimation of the discriminatory power (d_p) of the 1-P, 2-P and 3-P color vectors R, G, B.

The RGB vectors for cellulose fiber identification were measured experimentally and expressed as the percentage of the correctly identified unknown cellulose fibre samples as follows: the number of unknown samples for one measurement of d_p (%), 20; the number of unknown samples from one kind of fibre F1 to F4, 5; 3 images and scans (1 scan of a selected fibre per image) were made from each sample; 100 px were selected from each sample from a set of 3 images; 3 scans, with n_{px} between 861 and 3409, by statistical random selection. Six parallel measurement experiments were conducted (Table 8), each containing 20 unknown samples (Table 7).

RESULTS AND DISCUSSION

The cellulose fibre models F1 to F4 simulate the well-known, standardized practice of fibre identification by staining and subjective visual evaluation because it is complex enough for subjective standardised visual identification with some problems in correctness, accuracy, and lack of quantitative data (Table 1).

Two or more stains are recommended for reliable identification (Kutscha and Gray 1972) because of the complexity and fuzzy character of visual identification. There are uncertainties associated with visually distinguishing between cellulose fibres of types F2 and F3 and the identification of F4.

The model is also complex enough with regard to objective identification: the clouds of data (Fig. 1) and probability density functions (Fig. 2) meaningfully overlap.

Subjective evaluation

The four fibre types were stained according to standards with zinc chloride-iodine stain (Herzberg stain) and the triiodide Graf "C" stain. Their colors were visually assessed and described (Table 1). The verbal description of the observed colors of 4 kinds of fibres stained by Herzberg stain and Graf "C" stain was made according to standards (ČSN 1976; STN 1993; TAPPI 2008). The colors of the fibres used for the Herzberg stain were as follows: groundwood, brilliant yellow; pulp (Whatman and sulphate), dark purplish gray to deep reddish purple; and rag, brilliant purplish pink to vivid red purple (Table 1).

Table 1. Visual Assessment and Description of the Colors of Cellulose Fibres Stained by Zinc Chloride-Iodine Herzberg Stain and Triiodide Graf "C" Stain According Present Standards (ČSN 1976; STN 1993; TAPPI 2008) for Visual Assessment

Type of Fibre	Color stained by zinc chloride-iodine stain (Herzberg stain)	Color stained by Graff "C" stain
F1: Groundwood	from yellow to brilliant yellow	vivid, yellowish orange; brightly yellow
F2: Sulphate pulp	dark purplish gray to deep reddish purple; reddish purple	dark bluish gray to dusty purple; brown-purple
F3: Whatman	brilliant purplish pink to vivid red purple; wine colored	moderate reddish orange; wine colored or brown-red
F4: Rag fibres	brilliant purplish pink to vivid red purple; wine colored	moderate reddish orange; wine colored or brown-red

The observed colors of the fibres stained by Graf "C" stain were also in agreement with the terms recommended in the standards.

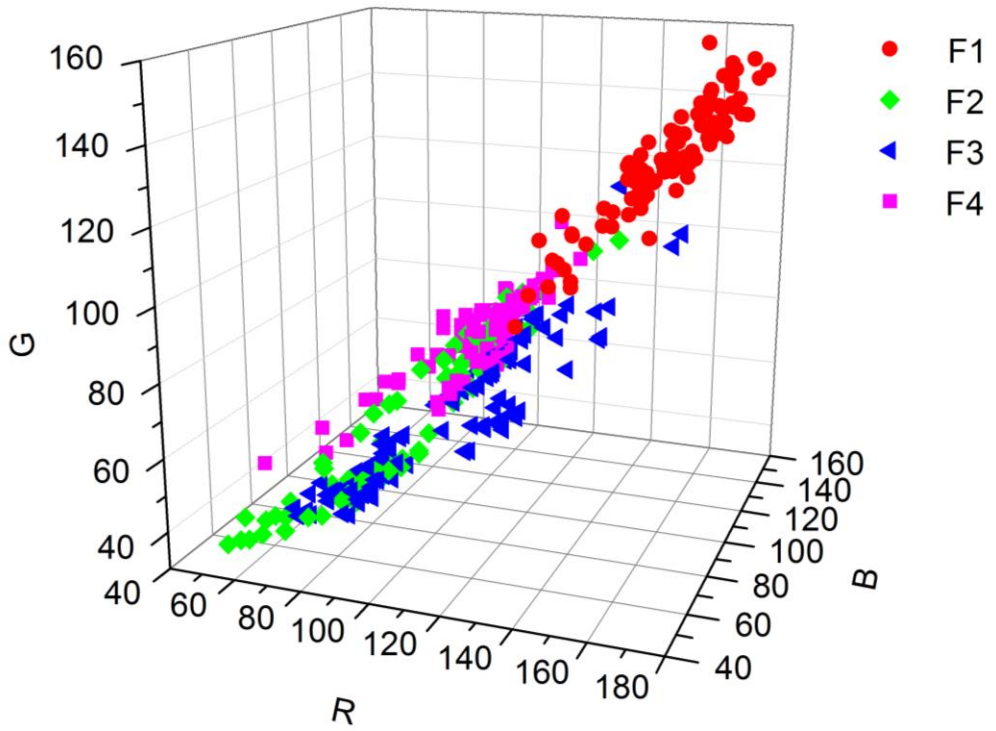
The subjective verbal color description has some drawbacks: (1) it is fuzzy; (2) it does not provide quantitative data that could be stored or processed; (3) no data are available for the continued improvement a database or optimization of the identification method; (4) the verbal information does not provide any information on saturation, excitation purity, chromaticity, or systematic information on lightness; (5) the fuzzy verbal description of categorical data cannot be used for valuable statistic evaluation; and (6) if one wanted to distinguish between the 4 kinds of fibres using subjective visual assessment and without use of morphological information, one stain was not enough, and it would be necessary to use at least 2 stains. The last drawback is that the testing time and cost, data volume, and complexity of objective identification would be large.

Figure 1 shows the overlapping 4 clouds of fibre types F1 to F4 of the R, G, and B vectors. Both the scatter plots (Fig. 1) and the probability density functions (Fig. 2) of the vector color data R, G, and B of the 4 kinds of fibres (F1, F2, F3, and F4) allow illustrating the differences of the color vectors in distinguishing between the combinations of fibre types F1/F4, F1/F2, F1/F3, F4/F2, F4/F3, and F2/F3.

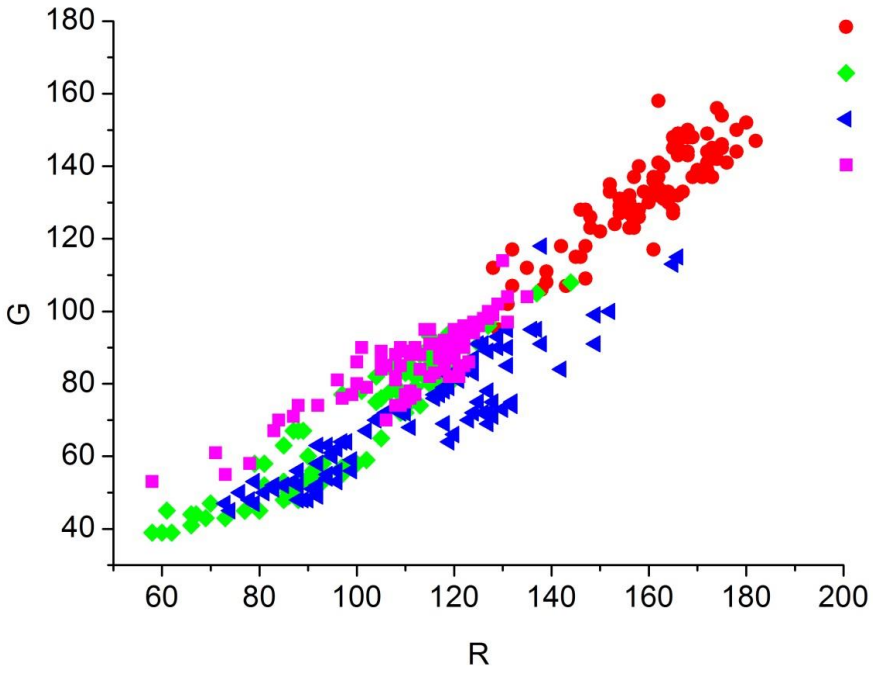
Staining by the Herzberg stain clearly revealed the differences between F1/F4 and F2/F4. The combination F2/F4 was hardly distinguishable using one dye.

One way of visualizing and estimating the d_p of 2 individual color vectors is using the area (U) of overlap of the two functions (Kraft *et al*. 2002). The overlapping area for the combinations F1/F4, F1/F2, F1/F3, F4/F2, F4/F3, and F2/F3 was relatively high, as shown in Fig. 2.

a)


F1
F2
F3
F4
G
R
B
160
140
120
100
80
60
40
180
200

b)


F1
F2
F3
F4
G
R
180
160
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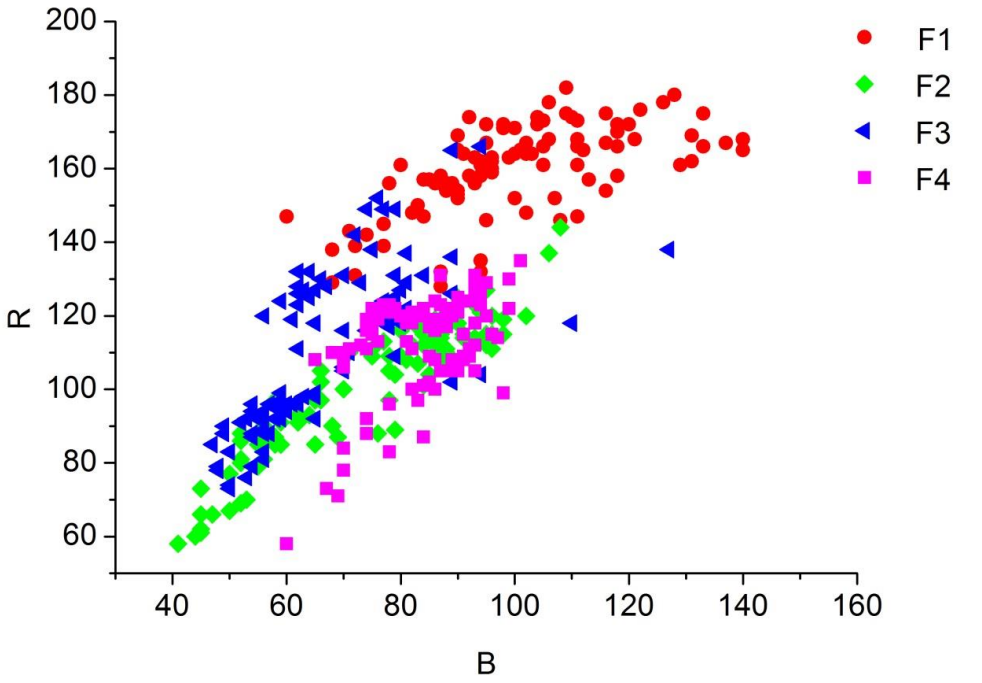
200

c)



d)

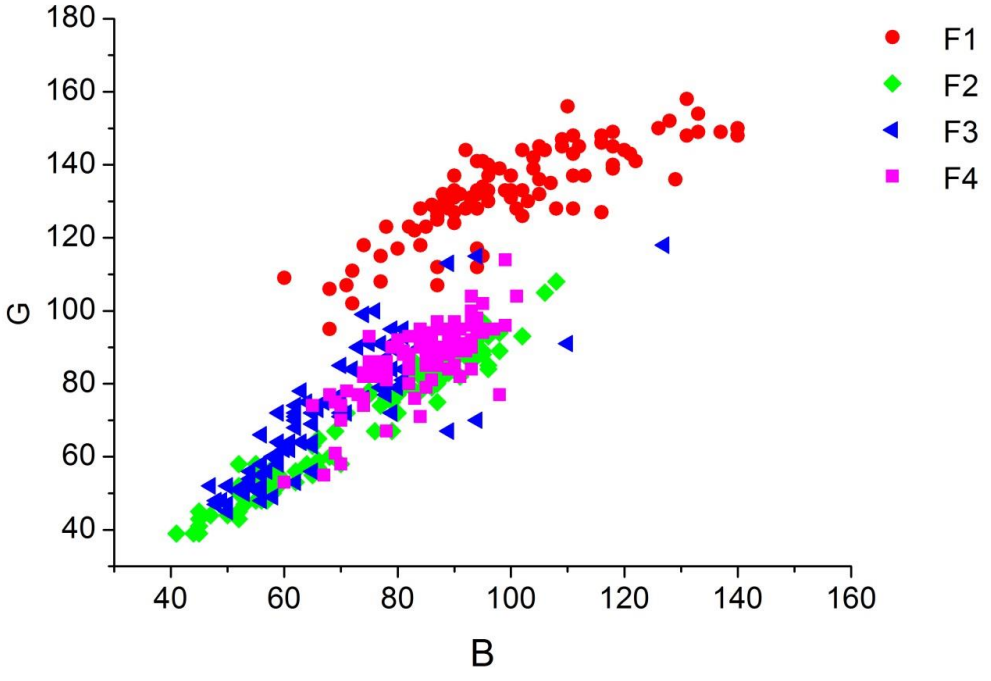
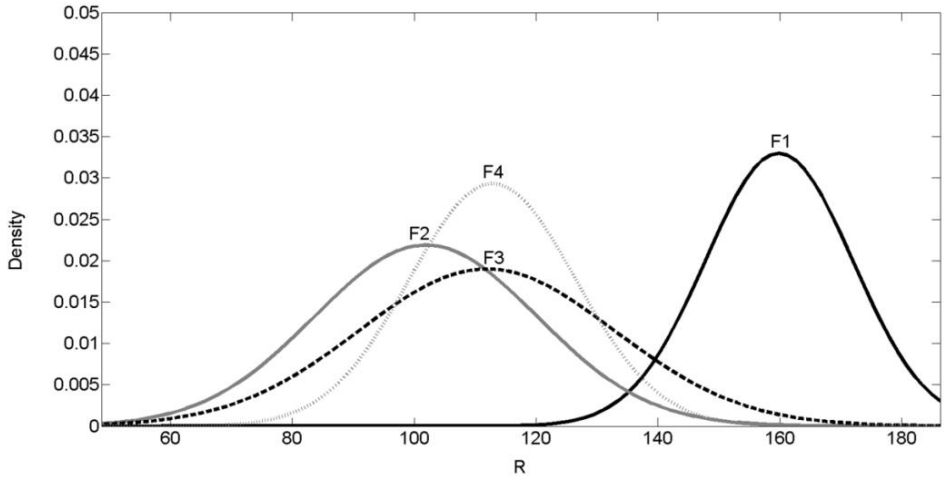
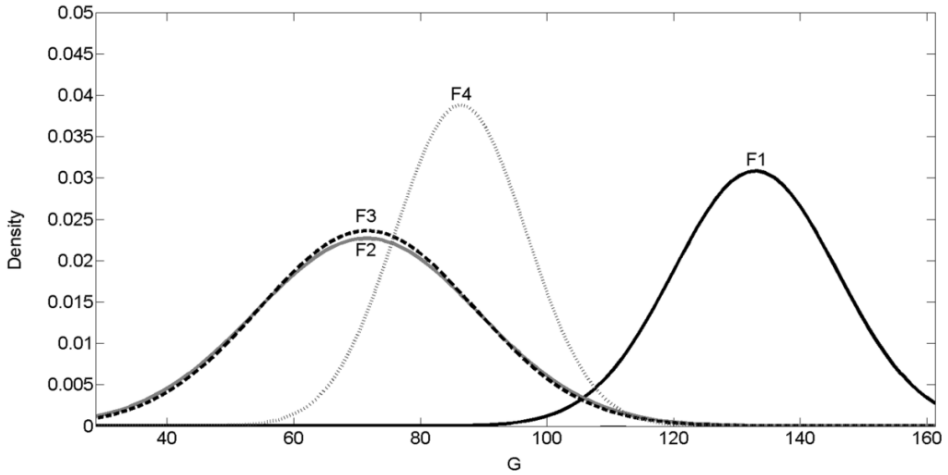


Fig. 1. The distribution of the R, G, and B vector parameters of the 4 fibre types stained by the Herzberg stain (the number of kinds of fibres, n_{fk} = 4; the number of R, G, and B pixels, n_{px} = 100), a) 3-P visualization and 2-P projections b) G-R, c) R-B and d) G-B projections

a) R



b) G



c) B

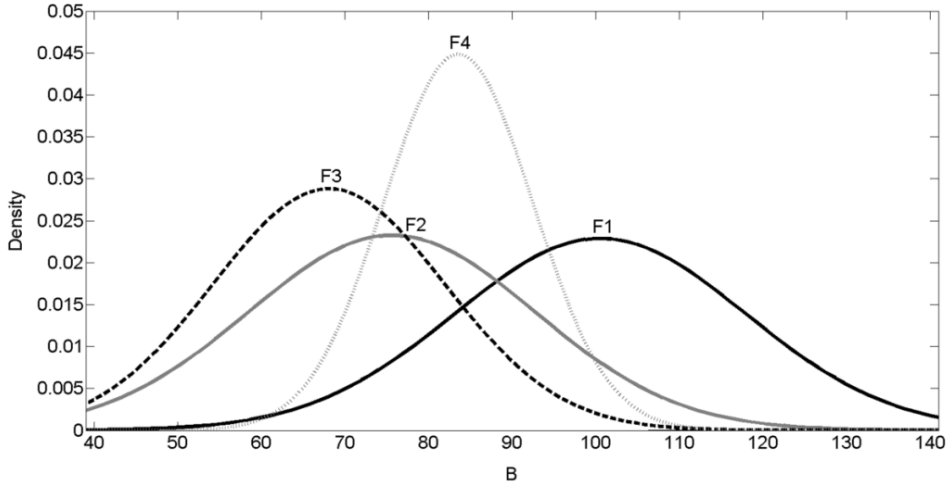


Fig. 2. Probability density functions of the CIE color vector parameters R, G, and B for 100 pixels of one scan of each of the four kinds of cellulose fibres: F1 (groundwood), F2 (sulphate pulp), F3 (Whatman paper), and F4 (rag)

Density plots (Fig. 2) are a much more effective way to view the practically normal distribution of a variable. Visualization *via* density plots also allows for better superimposing of the kernal density plots of the four groups of the vector color data, for better comparison of the overlapping of the densities, and the groups of color data for the 6 combinations of the 4 kinds of fibres for each color vector.

Other alternatives were considered to better distinguish between these 4 fibre groups. Discriminatory analysis and density plots, along with distinguishable degree, were applied in these alternatives.

The probability functions overlap significantly, meaning that identification is complex and it can be assumed that d_p is relatively small. It is not simple to visually or objectively discriminate between the colors of the 4 kinds of stained fibres. Another visualisation option, histograms, is neither suitable for determining the shape of a distribution nor for the comparison of groups of data for the 4 fibre kinds because it is so strongly affected by the number of bins used.

Internal Correlations and Dimensionality

The color of every pixel can be described using three vectors. These vectors are theoretically internally or mutually independent dimension variables. If the vectors are mutually correlating, it is a property of a measured material, object, or system and not of the R, G, and B quantities. This attribute, an extent of internal correlation of theoretically independent dimensions, may be characteristic for a given system (material, fibre kind, object, dynamic system).

Table 2. Internal Correlations Coefficients (R) between the R, G, and B Vectors of the Scanned Microimages of the 4 Fibre Types Stained by the Herzberg Stain

100 px	r_{R-G}	r_{R-B}	r_{G-B}
F1	0.91	0.79	0.85
F2	0.99	0.93	0.95
F3	0.78	0.39	0.78
F4	0.74	0.48	0.54
μ_r	0.85	0.65	0.78

The color vectors R, G, and B of the 4 cellulose fibre types used in the studied model are partially correlated. The highest internal correlations, close to r = 1, were found for the vectors R, G, and B in stained cellulose sulphate pulp fibres (F2).

Comparing Groups/Density Plots

Variance analysis and discriminatory analysis allow one to group data into classes based on observed parameters. Both of these methods, and several other statistical methods, stem from an assumption that the data observed satisfy a normal probability distribution. By applying multi analysis of variance (MANOVA), a hypothesis on the identity of the mean values of the four fibre types for 100 randomly chosen RGB color parameter values was statistically tested. The compared mean values were three parametric (3-P) vectors. The hypothesis that all four mean values were identical was subjected to testing where H_0: $\mu_{F1} = \mu_{F2} = \mu_{F3} = \mu_{F4}$ and H_1: at least one pair of mean values differs. This test determined whether or not the variability factor substantially influenced the values of the measured quantities R, G, and B.

Table 3. Mean Values of R, G, and B Parameters and their Standard Deviations for the 4 Types of Fibres

Type of fibre	R	σ_R	G	σ_G	B	σ_B
F1	160	12	133	13	101	17
F2	102	18	71	18	76	17
F3	112	21	72	17	68	14
F4	113	14	86	10	84	9

For RGB with the values χ^2 = 935.6, p-value = 0.0000, and α = 0.05, the null hypothesis H_0 was rejected. This means that the set was not homogeneous and at least one kind of fibre was distinguishable. Further, the distinguishability of all pairs F1/F2, F1/F3, F1/F4, F2/F3, F2/F4, and F3/F4 was tested using the Wilks (Λ) and the Chi-Square (χ^2) test (Table 3).

Table 4. Comparison of Differing Pairs of Fibres

Type of fibre	Λ	χ^2	p-value	F-ratio
F1/F2	0.09	474.53	0.00	665.68
F1/F3	0.19	435.93	0.00	535.30
F1/F4	0.13	403.49	0.00	443.88
F2/F3	0.61	96.08	0.00	41.20
F2/F4	0.57	111.57	0.00	49.94
F3/F4	0.41	173.53	0.00	92.67

The best distinguishability was for the pairs F1/F2, F1/F3, and F1/F4, which had the highest F-ratios. The lowest F-ratios were found for F3/F4, F2/F4, and F2/F3.

In using one-dimensional testing to distinguish the fibre types through the F-ratio and p-value (Table 5), it was found that all three parameters R, G, and B allowed distinguishability. Based on the ANOVA test, for one-parametric testing, the best parameter was G, followed by R and B.

Table 5. ANOVA of F-Ratios and P-Values for the Individual Color Vectors R, G, and B

Color parameter	F-ratio	p-value
R	244.03	0.00
G	389.75	0.00
B	89.09	0.00

In connection with this result, the following facts are interesting: (1) the human eye is the most sensitive to green; and (2) in the camera used and the majority of digital cameras, the recording of a color image is performed using a single CCD sensor (charge-coupled device) with color filters located directly within a scanner over each single pixel, containing more green pixels, usually 50%, while the other 50% is divided between red and blue (*e.g.*, Bayer (1976) array). This image sensor has a higher sensitivity to green color, which simulates the color sensitivity of the human eye (Neitz and Jacobs 1986). Such an arrangement is also applied in the camera used and the majority of digital cameras currently produced. The current Bayer filter design uses GRGB or RGGB filters.

Other types, such as the Kodak Truesense image sensor, also have higher sensitivity to green light.

As shown later (Tables 7 and 8), during the objective identification of fibres using individual vectors R, G, or B, the G vector led to the lowest percentage of correctly identified fibres. It is necessary to study the discriminatory power of the individual vectors, their combinations, and potentially derived characteristic quantities in more detail to identify cellulose and other kinds of fibres in the future.

Table 6 shows that, according to MANOVA, which is based on the statistical test procedure for comparing multivariate (population) means of several groups (Warne 2014), the percentage of correctly classified fibres for one-parametric (1-P) evaluation of R, G, B ranged between 46 and 57%. For two-parametric (2-P) evaluation (RG, RB, and GB) it was 63 to 70%. For three-parametric (3-P) evaluation, it reached 74%.

Table 6. MANOVA – Percentage of Correctly Classified Fibre Sample Picture Elements using RGB Parameters

1-P	%	2–P	%	3–P	%
R	50.50	**RG**	68.50	**RGB**	74.00
G	57.00	**RB**	63.00		
B	46.25	**GB**	69.50		

An increase in the number of parameters leads to an increase in the percent of correctly classified picture elements (RGB pixels) of the fibres.

Distinguishability of the Pairs of the Cellulose Fibre Kinds

The results of the variance analysis led to rejection of the null hypothesis (Table 3). This means that the data were not homogeneous and that the sets were mutually distinguishable to a certain extent.

The fundamental questions of distinguishability are as follows: first, what is the distinguishability of fibre types using color vectors and the vector combinations R, G, B, R/B, R/G, B/G, R/G, and R/G/B? Second, what is the difference in the distinguishability of the individual parameters of R, G, and B and their combinations in the identification of particular cellulose fibre types?

Quantifying the discriminatory power $\underline{d}_p$ can be done by numerous methods using the distance, overlapping area, or empirically (*e.g.*, by measuring the percentage of correct analytical decisions or identifications). Discriminatory power can be expressed as the distance between the centres of two groups (types of fibres) using the index of distinguishability, V_{ij}, defined as follows (Varga 2012) in Eqs. 1 and 2,

$$V_{ij} = \frac{d(S_i,S_j)}{\sqrt{(1+r_i)(1+r_j)}} = \frac{d_{ij}}{\sqrt{(1+r_i)(1+r_j)}} \quad (1)$$

where d_{ij} is a function of S_i and S_j and is the distance between the centres of groups i and j; r_i is the mean range in group i; and r_j is the mean range in group j.

$$V_{ij} = \frac{d(S_1,S_2)}{\sqrt{(1+r_1)(1+r_2)}} \epsilon < 0, \infty > \quad (2)$$

The properties of the distinguishability index are as follows: (1) $V_{ij} \epsilon [0, d(S_i, S_j)]$; (2) the higher the index V_{ij}, the higher the distinguishability between the particular pairs of fibre kinds and the discriminatory power in the identification of unknown fibres; and (3) the groups are excellently distinguishable if $V_{ij} > 1$; when V_{ij} is between 0.8 and 0.9, distinguishability is satisfactory; when $V_{ij} < 0.5$, the distinguishability of groups is less significant.

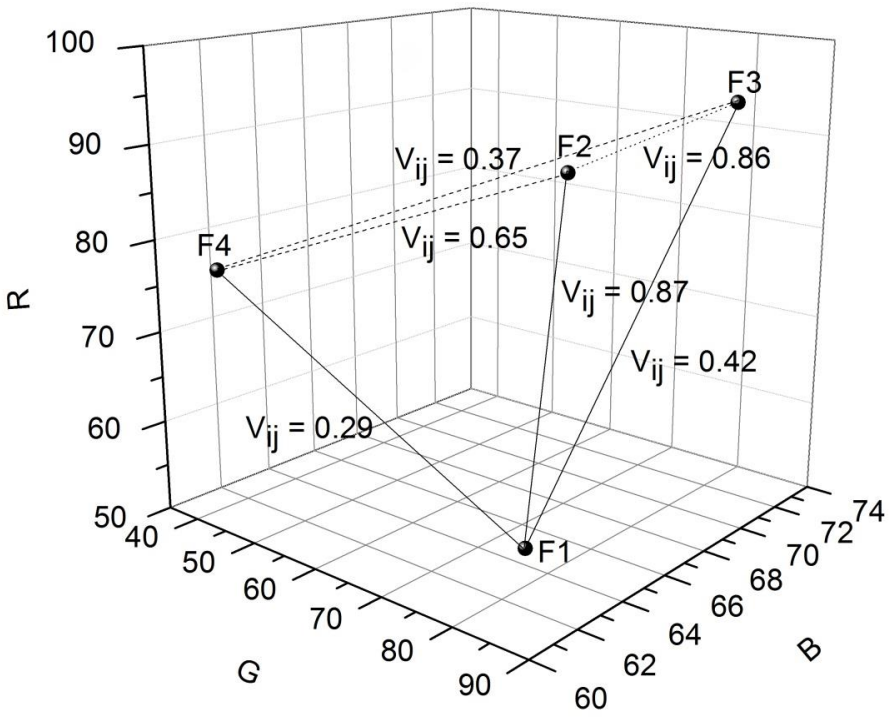


Fig. 3. Distinguishability indexes of RGB parameters in distinguishing between the picture elements of the four kinds of cellulose fibres F1, F2, F3, and F4

Based on the distinguishability indexes shown in Fig. 3, it is possible to reach a high resolution of the F1/F2, F2/F3, and F2/F4 (V_{ij} values of 0.87, 0.86, and 0.65, respectively) fibre pixels. Resolution of the other fibre combinations (F1/F3, F1/F4, and F3/F4) is more demanding.

The results shown in Figs. 1, 2, and 3 and Table 1 can be summarized as follows: in the model system comprising the 4 kinds of fibres F1 to F4, it is complex and difficult to visually identify the fibre type, especially when using one stain; the data clouds (Fig. 1) and density functions (Fig. 2 a-c) overlap and the overlapping area of the individual parametric color functions is high (Fig. 2); and the index of distinguishability between some of the pairs of cellulose fibre types is low (Fig. 3).

After answering the basic question of whether or not the 3 color dimensions R, G, and B are correlated, preliminary statistical evaluation of the discriminatory power and value of the R, G, and B vectors from each picture element, experimental verification of the objective method of fibre identification was done.

The problems with low distinguishability were solved by proper sampling and the averages of color vectors from a number of stained fibre microphotograph picture

elements of unknown samples were used. Discriminatory analysis of the average values of the R, G, and B vectors of the unknown sample, with the reference of the four kinds of the cellulose fibres, was used.

Experimental Verification of the Objective Method

Six parallel experiments comprising sample preparation and the identification of the unknowns were carried out (Table 8). Each experiment involved a set of 20 unknown samples, 5 of each type of fibre F1 to F4. On each fibre, the 7 x w_f pixel measured elementary area was marked, where w_f is the fibre width (px). The color of each unknown sample was represented by the mean of 100 px randomly selected from 3 fibres.

Table 7. Identification of 20 Unknown Fibre Samples (F1 to F4) by Discriminatory Analysis of the Color Vectors RGB from 100 Picture Elements (Px) from the Microphotography of the Fibres Stained by the Herzberg Stain

	Correctness of identification of unknown sample: 1 – correct, 2 – false							
	1-P			**2-P**			**3-P**	
No.	**R**	**G**	**B**	**RG**	**RB**	**GB**	**RGB**	**Correct answer**
1	0 (F4)	1	1	1	1	1	1	F3
2	1	1	1	1	1	1	1	F1
3	1	1	1	1	1	1	1	F3
4	1	0 (F2)	1	1	1	1	1	F3
5	1	1	1	1	1	1	1	F1
6	1	0 (F3)	1	1	1	1	1	F2
7	1	1	1	1	1	1	1	F4
8	1	1	1	1	1	1	1	F1
9	0 (F2)	0 (F2)	1	1	1	1	1	F3
10	1	1	1	1	1	1	1	F1
11	1	0 (F3)	1	1	1	1	1	F2
12	1	1	1	1	1	1	1	F1
13	1	0 (F3)	1	1	1	1	1	F2
14	1	1	1	1	1	1	1	F4
15	1	1	1	1	1	1	1	F4
16	1	1	1	1	1	1	1	F4
17	1	0 (F3)	1	1	1	1	1	F2
18	1	1	1	1	1	1	1	F4
19	0 (F2)	0 (F2)	1	1	1	1	1	F3
20	1	0 (F3)	1	1	1	1	1	F2
d_p (%)	85	60	100	100	100	100	100	
σ_{dp}	0.37	0.50	0	0	0	0	0	
v_{dp}	0.43	0.84	0	0	0	0	0	

Symbols: No. – unknown sample number; 1-P, 2-P, 3-P – number of parameters at identification; σ_{dp} – standard deviation; v_{dp} – coefficient of variation (%); d_p (%) – discriminatory power expressed as the percentage of correctly identified fibre samples.

Each parallel experiment involved the identification of the unknown samples using each of the 7 methods: 1-parameter identification using R, G, or B; 3 evaluation methods using 2-parameter identification using RG, RB, or BG combinations; and 1 method using 3-parameter RGB identification. The identification was made by the discriminatory analysis using averages of the R, G, and B color information for each unknown sample. An example of the identification of 20 unknown samples, 5 samples of each type of fibres F1 to F4, by the 7 methods is shown in Table 7.

In this identification experiment, the main cause of false identifications in columns R and G was insufficient distinguishing between the cellulose fibres F2 and F3. Of the total number of fibre type identifications (140), only 5 were false using one vector (1-parameter evaluation R) identification. The discriminating power, as expressed by the number of incorrect identifications, increased as follows: d_p (G) = 60%; d_p (R) = 85%; and d_P for the 2- and 3-parameter identification was 100%.

To verify whether or not objective identification using solely color information without morphological information was possible, the experiment in Table 7, involving 140 unknown fibre identifications, was repeated (Table 8). The first line in the Table 8 summarizes the results shown in Table 7, and each following line represents a parallel experiment. The total number of unknown fibre samples was 6 x 20 = 120 in each of the 6 parallel experiments described in Table 8. Each sample was identified *via* 7 methods, and the total number of verifying discriminatory analyses and fibre identifications was 120 x 7 = 840.

Table 8. The Average Percentage of Correctly Identified Unknown Samples using the R, G, or B Parameters in 840 Discriminatory Analyses of Fibre Identifications of the 120 Unknown Samples using the Three 1-P, Three 2-P, and One 3-P Identification Methods

No.	1-P			2-P			3-P
	R	G	B	RG	RB	GB	RGB
1	85	60	100	100	100	100	100
2	100	60	95	100	100	100	100
3	100	60	100	100	100	100	100
4	95	75	85	100	100	100	100
5	95	75	90	100	100	100	100
6	95	80	90	100	100	100	100
μ_{dp}	95	68	93	100	100	100	100
σ_{dp}	5	9	6	0	0	0	0

Symbols: No. – parallel experiment number; 1-P, 2-P, 3-P – number of parameters at identification by 1, 2, or 3 color vectors RGB; μ_{dp} – average discriminatory power; σ_{dp} – standard deviation

The discriminatory power (d_p), in terms of the percentage of correctly identified unknown samples, of the G vector was in the range 60 to 80%. Those of the color vectors R and B varied between 85 and 100%, and those of the 2-parametric and 3-parametric were 100% for the 100 px averages.

This objective micro-colorimetric method of fibre identification using scanned RGB data from the material digital photography successfully tested on the cellulose fibre model could also be applied to other kinds of fibre material identification.

Recommended procedure for the objective micro-colorimetric fibre identification

Based on the knowledge acquired regarding the objective micro-colorimetric fibre identification using RGB vectors from digital photography, the following procedure is recommended: (1) selection of fibre types that can be analysed in a given laboratory and sampling of the known fibre types for comparative calibration analysis (DB) should be of representative nature (ČSN 1976; AATCC 1990; STN 1993; ASTM 2007; TAPPI 2008; ASTM 2010); (2) defibration and microscopic staining preparation of the selected fibre types according to various standards (ČSN 1976; AATCC 1990; STN 1993; ASTM 2007; TAPPI 2008; ASTM 2010); the zinc chloride-iodine stain (Herzberg stain) used in this contribution can be delivered by the Institute of Paper Science Technology (Institute of Paper Science Technology 2009) or prepared in the laboratory according to standards (ČSN 1976; AATCC 1990; STN 1993; ASTM 2007; TAPPI 2008; ASTM 2010); (3) the microphotograph of the microscopic sample is made at 40x magnification and transferred to a computer; (4) a uniformly-stained, non-deformed fibre without bubbles or other defects is selected from the microphotograph by a cursor; a scan area (*e.g.*, 7 x w_f px, where w_f is the fibre width) is selected and the RGB data from each pixel are transferred to the database; (5) identification of the unknown sample by discriminatory analysis of the reference fibre and unknown fibre data; formation of a microscopic preparation, taking photographs and transferring the data of the unknown are to be performed in identical modes as those for reference fibres.

CONCLUSIONS

1. The objective identification method using R,G,B color vectors enabled the correct identification. No morphological information regarding the form of the fibre needed to be known; Experimental verification by the analysis and identification of 120 unknown samples was done; Up to 100% of the unknown samples were identified correctly using the individual color vectors R or B, 2-P (RG, RB, GB), or 3-P(RGB) combinations from the digital microphotographs of the stained fibres.
2. The model of cellulose fibres well-simulates the problems associated with subjective visual evaluation of fiber type: the visual identification of fiber types F1 to F4 is complex; the verbally estimated colors are fuzzy; 2 or more stains are usually needed for visual identification according to present standards; the color data clouds of the fibre types F1 to F4, as well as the density functions, strongly overlap; and the estimated distinguishability index (V_{ij}) is low.
3. The objective method of identification enabled reduction of the number of stains used for the visual identification from 2 to 1, which increases testing speed and decreases the data volume and costs of the objective fibre identification.

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